[redacted]
10/5/97
Aug -

# SURFACE MODIFICATION FOR BIOCOMPATIBILITY

11th Quarterly Report
Covering Period July 1 - September, 30 1997
Contract No: N01-NS-5-2321

James J. Hickman, Ph.D.
Science Applications International Corporation
Life Sciences Operation
1710 Goodridge Drive, MS 188
McLean, VA 22102

Submitted to:

Neural Prosthesis Program
Division of Fundamental Neurosciences
National Institute of Neurological Disorders and Stroke
National Institutes of Health
Federal Building, Room 9C02
Bethesda, MD 20892-9170

**THIS QPR IS BEING SENT TO YOU BEFORE IT HAS BEEN REVIEWED BY THE STAFF OF THE NEURAL PROSTHESIS PROGRAM.**

## TABLE OF CONTENTS

**Figure 1.** Phase-contrast photomicrographs of 1-day-old cultures of cortical precursor cells isolated from embryonic day 13 rats and grown in serum-free medium on poly-D-lysine, DETA and OTS. Initial attachment and survival was comparable on all substrates.

**Figure 2.** Phase-contrast photomicrographs of 3-day-old cultures of cortical precursor showing clusters of dividing cells on poly-D-lysine coated surface. In contrast only a few differentiated cells were present on DETA and OTS. This indicates that only those precursor cells grown on poly-D-lysine will divide in response to bFGF.

## PROJECT SUMMARY

The purpose of this project is to develop modified artificial surfaces for implantable biocompatible electrodes in the cerebral cortex. To this end, primary objectives of the project have been focused on the development of a defined *in vitro* cell culture model and to use *in vitro* cultures to select surfaces that could be used to manipulate neurons, astrocytes, and/or microglia in embryonic and adult stages of development. Dr Hickman presented a summary of our research at the Neural prosthesis Meeting in Bethesda in October. We will review our progress and findings in the final report due Dec. 1997.

The report for this quarter is brief as financial considerations have limited our efforts. This quarter we did no new experimentation and have instead been writing, doing data analysis and finalizing figures for the completion of manuscripts, and budgeting for rewrites or potential future experiments to meet reviewers' requirements.

We have completed the paperwork for the initiation of the proposed in vivo implantation studies, in collaboration with Sharon Juliano at USUHS, Bethesda, MD. (outlined below). However, we may not be able to move forward with these studies as a result of the need to channel limited funding toward completion of existing projects/ manuscripts.

We report some interesting results from experiments with cortical precursor cells cultured on poly-d-lysine, DETA, and OTS. This preliminary study provides evidence that cortical precursor cells can only be expandedwith bFGF on the poly-d-lysine surface. These cells did not proliferate on SAMs substrates tested.

## OVERALL PROJECT OBJECTIVES

a) Selecting candidate surfaces that are likely to enhance the microscopic mechanical stabilization of a microstructure implanted within the central nervous system/

b) Selecting candidate organic surfaces that are likely to enhance the close approximation of neurons or neuronal processes to specific regions of implanted silicon microstructures;

c) Developing or adapting available methods to bond the selected organic molecules to a silicon dioxide surface like the surface of a micromachined electrode (Tanghe and Wise: A 16-channel CMOS neuronal stimulating array (*IEEE Trans. Sol State Circuits* 27: 69-75, 1992) and to chemically characterize these surfaces before and after protein adsorption.

1. The attachment method shall be stable in saline at 37'C for at least 3 months;
2. To use silane coupling as the method of attachment;
3. To use the silanes to control the spatial extent (ie., the pattern) of the deposited surface.

d) Developing a cell culture or other suitable model of mammalian cortex and investigate the growth and adhesion of neurons, glia, micro-glia, and other cells present in the nervous system on substrates coated with the selected surfaces;

e) Cooperating with other investigators in the Neural Prosthesis Program by coating microelectrodes (estimated 50 over the contract period with the most promising materials for *in vivo* evaluation as directed by the NINDS Project Officer.

## BACKGROUND

Biomaterials that penetrate into the central nervous system as the microscopic electrode shafts of neural prostheses interact with neural and other tissues on a cellular and molecular level. In order to achieve tight coupling between these implanted microelectrodes and the target neural substrate, this interaction must be understood and controlled. Controlling the interaction requires an understanding of how cells, including neurons and glia, and extracellular proteins respond to the surface chemistry and any leachable substances of implanted biomaterials. This contract supported research will study these interactions with a long-term goal of rationally designing microelectrode surfaces to promote specific tissue interactions.

Presently, available clinical neural prosthetic implants typically use stimulus levels that excite volumes of neural tissue ranging from cubic

millimeters to cubic centimeters around the electrode. Because of the large stimulus intensities required, precise control of the response of neurons within the first few cell layers of an implanted electrode has not been necessary. Recent developments in the areas of micromachining and fabrication of silicon integrated circuit microelectrodes have introduced the possibility of controlled stimulation of smaller volumes of neural tissue--on the order of one thousand to one hundred thousand times smaller than those used today.

The efficiency of these microelectrodes depends on the micro-environment around stimulating sites. The surface of the microelectrodes and the proteins that adsorb to this surface have a major impact on the way in which different cell populations react to the implant. Neural growth cones are sent out from many neurons around a microelectrode following implantation. With appropriate surfaces, it may be possible to get selected neurons to send processes directly to the microelectrodes. Glia and other cells also respond to an implanted electrode. With appropriate surfaces it may be possible to promote cell adhesion and anchoring of some areas of the implant structure while leaving other areas with minimal response from glial cells. This study will investigate cellular and molecular responses to specific surface modifications of silicon microelectrodes.

A meeting on July 18th with the NIH program project manager has led us to focus on *in vivo* study of surface-modified electrodes for the remainder of the contract period (August to December 1997). We will modify an electrode surfaces with self-assembled monolayers (SAMs) and, possibly, with biological macromolecules. The biocompatibility of these stimulating electrodes will be evaluated after implantation in the rat cerebral cortex.

The animal surgery and post-operational care will occur at Uniform Services University of the Health Sciences, Bethesda, with Dr. Sharon Juliano. Tissue immunocytochemistry and analysis will be carried out at SAIC, Biotech Applications Division, Taft Ct.

Scope of proposed in vivo experiments

The goal of this contract is to design interfaces to examine and control the interaction of surfaces with biological systems both *in vitro* and, ultimately, *in vivo*. We have used self-assembled monolayers, in conjunction

with biological macromolecules; to coat electrode materials to render them biocompatible for cell culture. However, it is not clear whether these newly-designed interfaces will control the interaction of the surfaces with neural cells in the cerebral cortex. Therefore, we will proceed with the following *in vivo* experiments as they were originally planned:

We will implant surface-modified iridium electrodes in the parietal cortex of rats. About 24 adult Sprague-Dawley rats are needed. Surgery will be performed by Wu Ma who is experienced in animal brain surgery. Postsurgical care will be provided by SAIC staff in USUHS animal facility. Rats are observed twice daily during the week and once daily on weekends to ensure uneventful recovery from anesthesia and surgery; to administer supportive fluids, analgesics, and other drugs as required; to provide adequate care for surgical incisions; to empty the animal's bladder by manual compression 2 times a day for 7-10 days; and to maintain appropriate medical records. Animals will be housed individually for 12 hours, 1, 7, 14 days and 1, 2, 3 and 4 months respectively before sacrifice. The rats will be killed quickly and humanely by overdose with sodium pentobarbital (80 mg/kg). The brain will be fixed with 4% paraformaldehyde in 0.1 M phosphate buffer by vascular perfusion via the left heart ventricle in a perfusion hood. Brain sections will be cut serially in 12-20 μm on a cryostat, thaw-mounted onto gelatin-coated slides. Implant surface characterization using morphology, XPS, immunostaining will occur at SAIC, Taft Ct. We will examine: histological and cell- phenotypic characteristics; neuronal and glial responses to surface-modified implants; extracellular proteins---Immunolocalization of macromolecules surrounding implants; and cytokines released by astrocytes, microglia, macrophages and neurons before and after implantation.

## RESULTS

### CELL CULTURE

Cortical precursor cells

In previous studies, we have shown that organosilane surfaces affect neurotransmitter phenotype expression in E 14, early embryonic cultures (see 9th Quarterly Report). However, it is not known how the substratum affects neural progenitor cell growth. A Neural precursor cell is an undifferentiated

cell that is characterized by its proliferative capacity and multipotency and located not only in embryonic brain, but also in the subventricular zone of the adult cerebral cortex. Recently, precursor cells from the embryonic cerebral cortex have been isolated and expanded *in vitro* using growth factors such as basic fibroblast growth factor (bFGF) (for review, see Temple and Qian, 1995; Johe et al., 1996). These cells become mitotically active via wounds created during surgical implantation of stimulating electrodes, and form a glial scars, which surrounds the electrodes and makes them ineffective. It is important therefore too address whether or not surface modification with SAMs may control glial scar formation and how cortical progenitor cells respond to organosilane surfaces.

We isolated precursor cells from the telencephalic neuroepithelium of embryonic day 12 and 13 rats in serum-free medium and expanded with bFGF. Timed pregnant Sprague-Dawley rats (Taconic Farms, Germantown, NY) were anesthetized with sodium pentobarbital (40mg/kg body weight, ip). The pups were removed from the dams and placed into Hank's buffered saline solution (HBSS). The embryonic age was determined by measuring the crown-rump length. The telencephalic neuroepithelium was dissected from rats of embryonic days 12-13. Dissected areas corresponded to the formative dorsal telencephalon according to the atlas of the prenatal rat brain by Altman and Bayer (1995). Tissue was dissociated by brief mechanical trituration in HBSS. The cells were collected by centrifugation and resuspended in a serum-free medium containing MEM/N3, glutamine and 10 ng/ml of recombinant human basic FGF (Intergen, Purchase, NY). Cells ($1 \times 10^6$) were plated on coverslips in 35 mm plastic dishes. The coverslips were precoated with either poly-L-lysine (20 ug/ml), a hydrophilic surface SAM, DETA, or a hydrophobic surface SAM, OTS. The bFGF was added daily, and the media changed every 2 days.

Pulse-labeling with BrdU was used to detect proportions of precursor cells synthesizing DNA during the cell cycle. Four hours before fixation, 10 μM BrdU (Sigma, St Louis, MO) was added to the culture medium. Cultures were washed and fixed with 2% paraformaldehyde and then 70% ethanol, and DNA was denatured by 2 N HCl. The cultures were washed in PBS and incubated with FITC-conjugated mouse-anti-BrdU monoclonal antibody (diluted 1:25 in PBS, Becton Dickinson, Mountain View, CA).

Cell survival was assessed by a comparison of the number of cells surviving at a given point relative to the number of cells survival at the time of the culture initiation, i.e., 2 hr. Cell survival is expressed as a percentage.

Results showed that at day 1, a few cells survived on these surfaces. Cell survival was similar on all three substrates, poly-D-lysine, DETA and OTS. However, at day 3, clusters of undifferented cells labeled by BrdU (not shown) were present on poly-D-lysine, but not on DETA and OTS. These results indicate that neural precursor cells dissociated from rat telencephalic neuroepithelium can be expanded by basic fibroblast growth factor on poly-D-lysine, but not on DETA and OTS.

## PAPERS IN PREPARATION

Regeneration of Axonal Processes of Adult Cortical Neurons, in vitro. Coulombe, M., W. Ma, Sathanoori, R. and J.J. Hickman

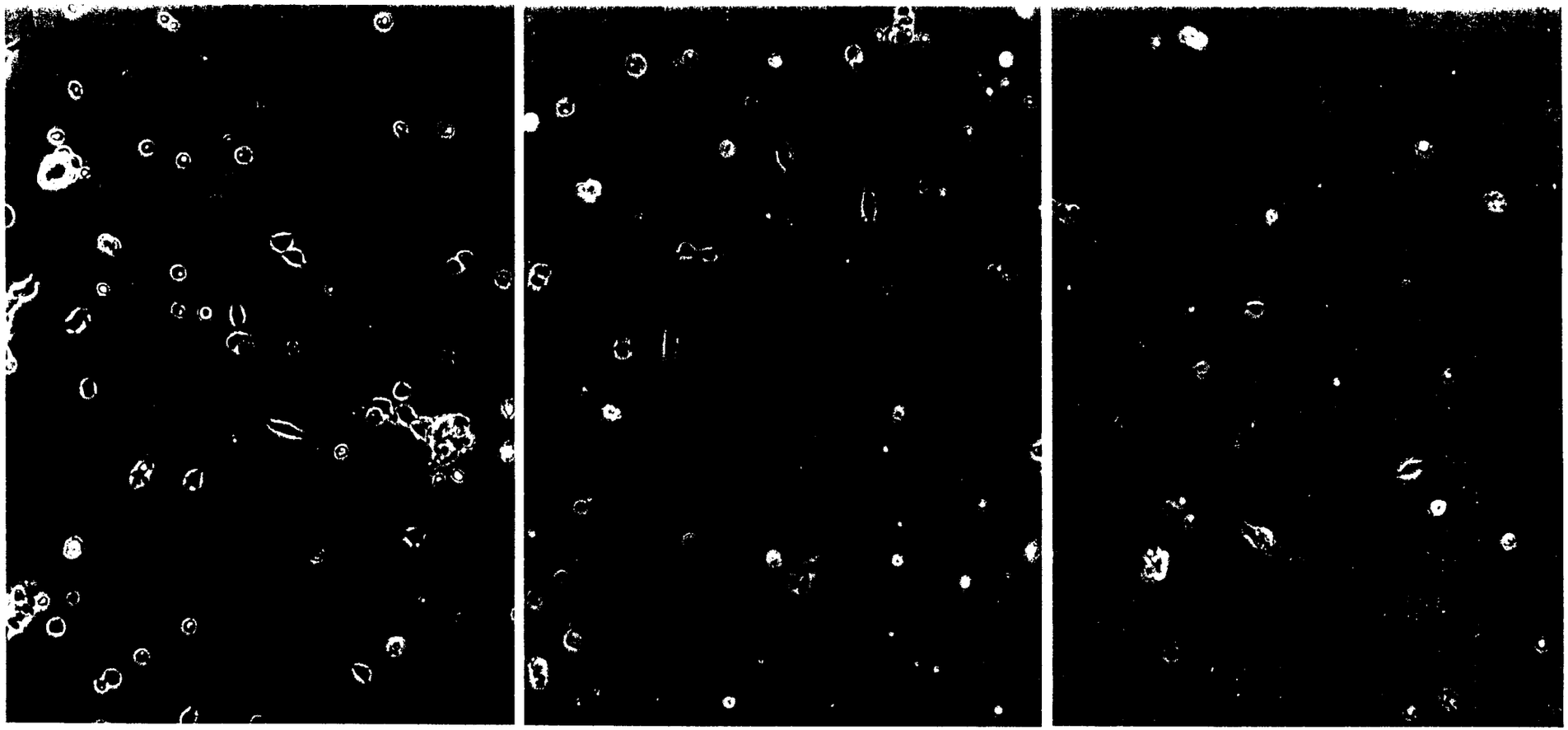
Hickman, J. J., Coulombe, M. G. and W. Ma. Differential Response of Astrocytes to Surfaces, bFGF and Implications for Implants.

Hickman, J. J., Jung, D. R. and M. Coulombe. Correlation of Cortical Neuronal Morphology to XPS Analysis of Substrate Materials.

Jung, D. R., Coulombe, M. G., Bateman, K. F., Sathanoori, R. S., Shaffner, A. E., Barker, J. L, Stenger, D. A and J. J. Hickman. XPS analysis of protein layers deposited by in vitro neuronal cell cultures.

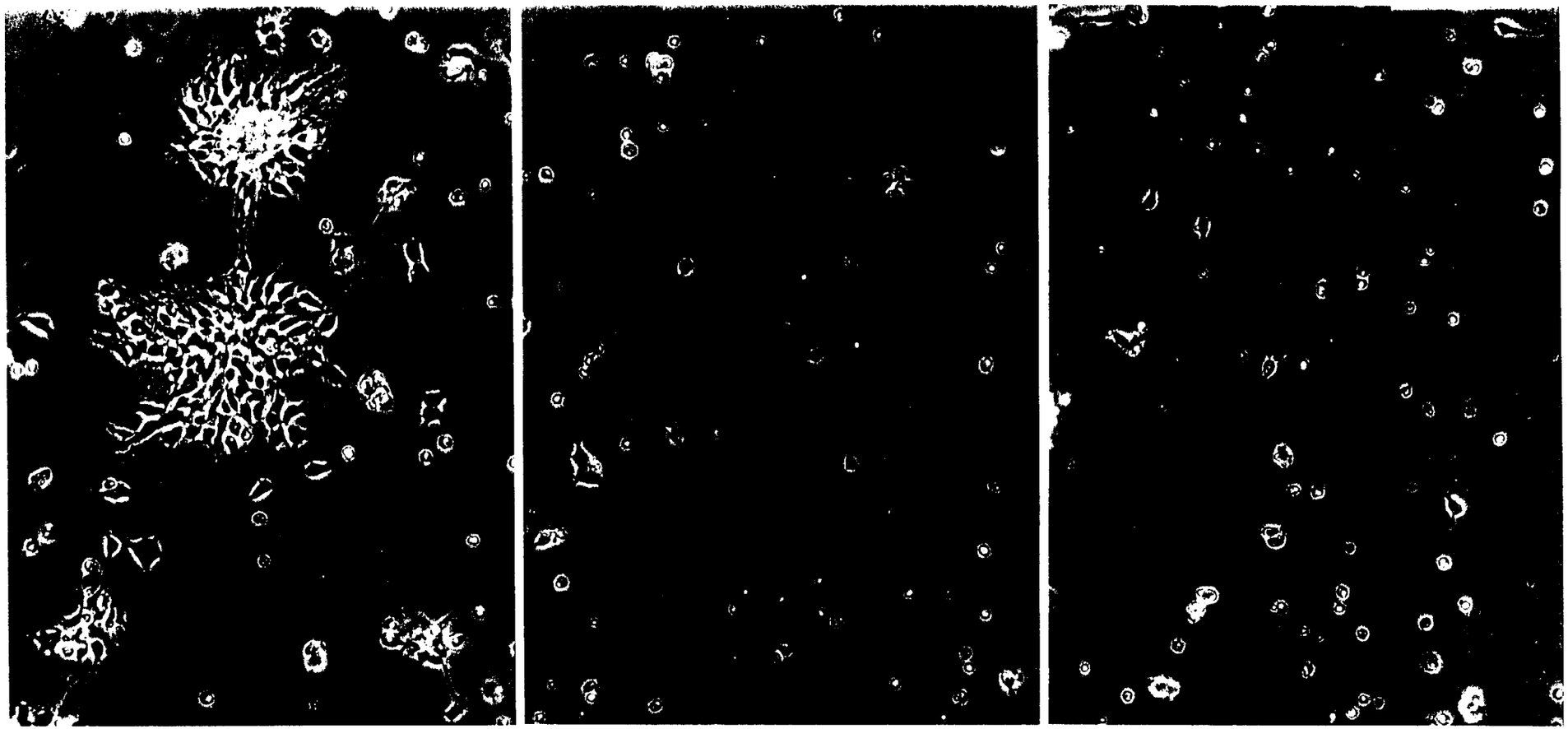
Ma, W., Coulombe, M. G., Jung, D. Sathanoori, R. and J. J. Hickman. Selective Expression of GABAergic and Glutamatergic Neurons on Organosilane Surfaces.

Cortical precursor cells on biological and SAM surfaces, 1 day in culture



PDL DETA OTS

Cortical precursor cells on biological and SAM surfaces, 3 days in culture



PDL DETA OTS